These phenomena may be the result of antibody combination with excreted metabolic products adhering to the surface of the trypanosome bodies. Further work on the protective power of antimetabolic-products serum, with or without absorption, is in progress.

It is probable that ablastin acts specifically against an enzyme or enzymes concerned in the nutrition of the parasites, as was postulated by one of us (*3, 5*) when the similarity between inhibition of reproduction of *Trypanosoma lewisi* and the interference with growth, development, and reproduction of the nematode *Nippostrongylus muris* was pointed out.

We believe that this production of functional immunity through the agency of antibodies directed against metabolic products, which interferes with nutrition, will be found to be a widespread phenomenon among infectious organisms (*6*).

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References and Notes

1. W. H. Taliaferro, *J. Exptl. Med.* 39, 171 (1924); ———, *Am. J. Hyg.* 16, 32 (1932).
2. E. A. Barnes, *Iowa State Coll. J. Sci.* 26, 1 (1951).
3. A. C. Chandler, *Am. J. Hyg.* 22, 157 (1935).
4. D. L. Augustine, *Proc. Am. Acad. Arts Sci.* 75, 85 (1943).
5. A. C. Chandler, *Am. J. Hyg.* 26, 309 (1937).
6. This work was supported by a research grant from the National Institutes of Health.
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Chromatographic Microassay for Cholesterol and Cholesterol Esters

Free cholesterol is readily separated from its esters by column chromatography on silicic acid. The separation is quantitative, and the two components can then be assayed separately. The procedure described here (*1*) requires relatively small samples and can be completed in less than 1 hour. With a simple modification of the extraction procedure, it is possible to get a reliable assay using only 0.02 ml of human plasma or 0.05 ml of rat plasma. The 100-mesh silicic acid sold by Malinckrodt especially for chromatography is satisfactory for this purpose. Ordinary reagent-grade products may be activated by heating them at 120°C overnight (*2*) and then quickly sieving them once in a moist atmosphere. The activated silicic acid must be protected from atmospheric moisture.

For quantitative assay, we use a chromatographic tube made of 8-mm (outside diameter) glass tubing about 25 cm long. The tube is abruptly constricted on one end to a diameter of 2 to 3 mm inside, and the end is drawn out about 10 mm to form a short nipple. To prepare the column, ram a small pledget of cotton down the tube to plug the constricted end. Fill the tube to a height of about 5 cm with silicic acid by means of an eye dropper and pack it by applying air at a pressure of 7 to 10 lb/in.^{2} to the top of the column. Prewet the column by forcing through about 2 ml of petroleum ether by means of the same air pressure.

For the routine assay of human plasma or serum, our procedure is as follows. Mix 0.1 ml of plasma or serum with 5 to 7 ml of 1/1 methanol and acetone. Bring the mixture to boiling, cool, dilute to 10 ml with solvent, and filter. Evaporate a 3.0-ml aliquot by placing the tube in cold water and bringing the water to boiling. Invert the tube while it is cooling to drain out the residual vapor of the solvent. Transfer the lipid to the column with three 2-ml portions of petroleum ether (boiling range 30° to 60°C) by means of an eye dropper.

Rinse the test tube and the chromatographic tube with each portion of solvent and force each through in turn. The flow rate should never be greater than 4 ml/min with any of the solvents. Care should also be taken never to allow the solvent level to go below the top of the silicic acid in this step and in the next one. At this point the free and esterified cholesterol are adsorbed at the top of the column. (This may be demonstrated by extruding the column by means of air pressure gently applied to the bottom of the tube and streaking it with concentrated sulfuric acid).

Develop the column with about 4 ml of 1/1 chloroform and petroleum ether. Collect this eluate, which contains the esterified cholesterol, in a separate test tube. Rinse the outside of the tip of the chromatographic tube with about 1 ml of petroleum ether, allowing the ether to run into the same test tube. To remove the free cholesterol from the column, elute with 4 ml of 1/1 methyl or ethyl acetate and petroleum ether. Force this through until flow ceases, and rinse the tip as before. Extrude the column by means of air pressure as described in the preceding paragraph and discard the silicic acid.

Evaporate the solvents in the aforementioned manner and take the fractions up in 3.0 ml of glacial acetic acid. Warm the tubes containing the esters to effect solution. Cool and add 2.0 ml of the ferric chloride-sulfuric acid reagent of Brown *et al.* (*3*) and mix thoroughly. Read the optical density at 560 mμ in the Coleman universal spectrophotometer. For precise work, it is necessary to prepare chromatographed solvent blanks. The standard contains 50 μg of cholesterol in 3.0 ml of acetic acid, and the blank for this is not chromatographed. The color develops rapidly and is stable for hours. With rat plasma or serum, an 8-ml aliquot of the methanol-acetone extract is required for a satisfactory reading of optical density.

Table 1. Estimation of free and stearyl cholesterol in mixtures containing different amounts.

Mixture of free and stearyl cholesterol chromatographed (mg of each)	Stearate found		Free cholesterol found	
	(mg)	(%)	(mg)	(%)
2.00	2.07	103.5	2.07	103.5
0.200	0.207	103.5	0.201	100.5
0.020	0.0201	100.5	0.0199	99.5

This procedure separates plasma cholesterol into its free and esterified fractions. If a plasma extract, or a mixture of cholesterol and its stearate, is placed on the column and the development with chloroform and petroleum ether is continued beyond the usual amount, it is found that the first two milliliters of eluate contain a large amount of cholesterol. This is esterified cholesterol. The third milliliter of eluate contains only a trace of cholesterol, and the fourth to about the tenth milliliters have none. As the development is continued beyond the tenth milliliter, the free cholesterol appears in the eluate. It is all eluted after about 20 ml of chloroform and petroleum ether have been used.

In Table 1 we see that the column will function satisfactorily over a very wide range of cholesterol or cholesteryl stearate concentration. In Table 2, evidence is presented that free cholesterol or its stearate added to an extract of plasma can be quantitatively demonstrated. Rat adrenal cholesterol is assayed by extracting the macerated gland in a hot mixture of methanol and ace-

Table 2. Recovery of free and of stearyl cholesterol which were added to plasma extract. The amounts added and found are given in milligrams per 100 ml of plasma.

Compound	Amount		Recovery (%)
	Added	Found	
Stearyl cholesterol	150	151	101
Free cholesterol	100	104	104

tone, evaporating a suitable aliquot, and chromatographing in the same manner. However, since the free cholesterol in the adrenal gland is relatively very low, one must use a large aliquot and dilute the ester fraction after chromatography if the free cholesterol level is desired.

Although the ester fraction can be determined by using the method of Brown *et al.* (*3*), the free adrenal cholesterol is accompanied by turbidity, which interferes slightly with this method. Turbidity is not observed with a modified Lieberman-Burchard procedure. Because of its sensitivity, the modified Tschugaeff reaction of Hanel and Dam (*4*) may be better suited to the determination of free cholesterol in adrenal tissue after chromatography on silicic acid.

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References and Notes

1. This work was supported in part by research grant No. C-2237 from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service.
2. W. Trappe, *Biochem. Z.* 305, 150 (1940); B. Borgstrom, *Acta Physiol. Scand.* 25, 111 (1952).
3. H. H. Brown *et al.*, *Anal. Chem.* 26, 397 (1954). The reagent is prepared as follows: dissolve 1.0 g of ferric chloride hexahydrate in 10 ml of glacial acetic acid. To 1.0 ml of this solution add 15 ml of chemically pure concentrated sulfuric acid, mix thoroughly, and dilute to 100 ml with sulfuric acid.
4. H. K. Hanel and H. Dam, *Acta Chem. Scand.* 9, 677 (1955).

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Actions of d-Lysergic Acid Diethylamide and Its 2-Bromo Derivative on Heart of Venus mercenaria

Physiological evidence indicates that the inhibitor nerves of the heart of the mollusk, *Venus mercenaria,* are cholinergic in nature (*1*), and that the activity of the excitor nerves is mediated by 5-hydroxytryptamine (5-HT, serotonin) (*2*). *d*-Lysergic acid diethylamide (LSD) was found to be an antagonist of serotonin on certain mollusk hearts under the conditions of the experiments (*3*). An early report that LSD was an effective antagonist on the *Venus* heart was later modified (*4*) when it became clear that LSD had a marked excitor action on this heart. This action persisted for long periods of washing during which a maximum amplitude of beat obscured the action of large doses of serotonin. It was stated that on the *Venus* heart, LSD acts as an essentially irreversible analog of serotonin (*4*). Recently, Shaw and Woolley (*5*) confirmed this observation.

The importance of a proper understanding of the fundamental mode of action of LSD prompts us to report further our earlier studies with LSD and our more recent observations of the action of 2-bromo-lysergic acid diethylamide (Bol-148, bromo-LSD) on the *Venus* heart (*6*). At a concentration of $10^{-6}M$, both serotonin and LSD produce a nearly maximal increase in amplitude in less than 10 minutes. After a heart has been washed for a few minutes, it recovers from serotonin; but after many hours of washing a heart that has been treated with LSD may still be greatly excited. No way has yet been found, including washing at a high *p*H, to restore quickly an LSD-excited heart. At concentrations below $10^{-9}M$, serotonin seldom excites the isolated *Venus* heart. If hearts are allowed to remain in a 10-ml bath of $10^{-10}M$ LSD, they are maximally excited in 1.5 to 2 hours. At a $10^{-16}M$ concentration of LSD, up to 3 hours may be required for the heart to adsorb an amount of LSD that produces near maximal excitation. Axelrod *et al* (*7*), from studies of tissue distribution, calculate that LSD exerts its characteristic effect in man at a level of 0.0003 μg/g of brain tissue. The *Venus* heart responds maximally at a tissue concentration that must be below this, for 10 ml of $10^{-16}M$ LSD contains only 602,000 molecules.

An important problem not yet resolved is whether the "LSD psychosis" results from central blocking of serotonin, or from a serotoninlike action of LSD, or for other reasons. 2-Bromo-lysergic acid diethylamide may prove useful in helping to solve this problem. Cerletti and Rothlin (*8*) found bromo-LSD to be a more effective antagonist of serotonin than LSD at a number of sites in mammals. This blocking action was highly specific, and they saw no signs of antihistamine, antiadrenaline or antiacetylcholine action. Certain of these observations have been amply confirmed and extended (*9*). Cerletti and Rothlin, however, failed to find any indication of an abnormal psychic disturbance produced by doses of bromo-LSD even 20 times as great as effective doses of LSD. They concluded that their results with bromo-LSD make it difficult to correlate the psychic effects of LSD with its antiserotonin property. The interesting observation has now been made by Ginzel and Mayer-Gross (*10*) that bromo-LSD, when it is administered 1 or 2 days before LSD, abolishes or greatly reduces the LSD psychosis without, by itself, having significant central action even in 2- to 3-mg amounts.

On the *Venus* heart, bromo-LSD is an effective antagonist of serotonin. On some hearts, high concentrations (10^{-4} to $10^{-5}M$) have a weak stimulating action resembling that produced by LSD, while on others there is no apparent effect. However, after treatment of hearts with bromo-LSD in concentrations in the range of 10^{-4} to $10^{-6}M$ for 1 hour or longer, the excitor action of a molar concentration of serotonin one-tenth as great is completely blocked. It is of further interest that previous exposure of a *Venus* heart to bromo-LSD abolishes or greatly reduces the excitor action of LSD that is subsequently applied. For example, on some hearts, pretreatment with $10^{-4}M$ bromo-LSD may completely prevent the otherwise marked excitor action of $10^{-6}M$ LSD.

Serotonin appears to be a normal regulatory neurohumor of the *Venus* heart. This heart is extremely sensitive to LSD, which has an excitor action resembling that of serotonin. Unlike serotonin, however, the action of LSD is very slowly reversed by washing. Bromo-LSD antagonizes the actions of both serotonin and LSD on the *Venus* heart. These several actions and interactions appear to parallel rather closely those seen in the mammalian central nervous system.

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References and Notes

1. C. L. Prosser, *Biol. Bull.* 78, 92 (1940).
2. J. H. Welsh, *Arch. exptl. Pathol. Pharmakol.* 219, 23 (1953).
3. ———, *Nature* 173, 955 (1954); *J. Marine Biol. Assoc. United Kingdom* 35, 193 (1956).
4. J. H. Welsh, in *The Hormones,* G. Pincus and K. V. Thimann, Eds. (Academic, New York, 1955), chap. 3.
5. E. Shaw and D. W. Woolley, *Science* 124, 121 (1956).
6. This work was supported by research grant B-623 from the National Institute of Neurological Diseases and Blindness, National Institutes of Health. We are indebted to R. Bircher of Sandoz Pharmaceuticals for supplies of *d*-lysergic acid diethylamide and its 2-bromo derivative.
7. J. Axelrod *et al.*, *Nature* 178, 143 (1956).
8. A. Cerletti and E. Rothlin, *ibid.* 176, 785 (1955).
9. L. Sollero, I. H. Page, G. C. Salmoiraghi, *J. Pharmacol. Exptl. Therap.* 117, 10 (1956).
10. K. H. Ginzel and W. Mayer-Gross, *Nature* 178, 210 (1956).

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Technique for Behavioral Analysis of Human Observing

The monitoring of a display (for example, a search radar) by human beings raises problems of considerable practical and theoretical interest. In general, the probability of detection of a signal varies directly with the signal rate; is a function of the temporal arrangement of the signals; and, in the case of low signal rates, varies inversely with the duration of the monitoring task. Such monitoring situations are badly in need of a descriptive behavioral analysis that would permit isolation of the variables which control the behavior underlying the probability of signal detection.